# Prevalence and diversity of extended-spectrum β-lactamases in faecal *Escherichia coli* isolates from healthy humans in Spain

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## Abstract

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates were detected in seven of 105 faecal samples from healthy humans, from two Spanish cities, during 2007. In these isolates, five ESBLs were detected, CTX-M-14 ($n$ = 2), CTX-M-1 ($n$ = 2), CTX-M-32 ($n$ = 1), CTX-M-8 ($n$ = 1) and TEM-52 ($n$ = 1). Both $bla_{\text{CTX-M-14a}}$ (surrounded by IS*Ecp1*-IS*903*) and $bla_{\text{CTX-M-14b}}$ variants (included in an integron structure) were identified in this study. This is the first time that the $bla_{\text{CTX-M-8}}$ gene and ESBLs of the CTX-M-8 group have been found in Europe and Spain, respectively. Faecal *E. coli* of healthy humans therefore constitute a reservoir of $bla_{\text{CTX-M}}$ genes with different surrounding genetic elements.

**Keywords:** CTX-M, *E. coli*, ESBL, faecal samples, healthy humans, Spain

**Original Submission:** 30 July 2008; **Revised Submission:** 1 September 2008; **Accepted:** 6 September 2008
Editor: R. Canton
**Article published online:** 9 June 2009

*Clin Microbiol Infect* 2009; **15:** 954–957
10.1111/1469-0691.2009.02803.x

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A great increase in the prevalence of extended-spectrum β-lactamases (ESBL) among clinical *Escherichia coli* isolates has been observed worldwide in the last few years, with CTX-M-producing *E. coli* especially prevalent as a cause of community-acquired infections [1,2]. Faecal carriage of ESBL-positive *E. coli* isolates has been reported in community and hospital patients in only few studies [3–6]. In addition, ESBL-positive *E. coli* isolates have also been found among healthy and sick animals and also in food in various countries [7–10], suggesting its spread in different ecosystems. Relatively few data exist thus far on faecal colonization by ESBL-positive *E. coli* isolates in healthy humans [4–6,11–13]. The objective of our study was to characterize and determine the prevalence of ESBL genes in faecal *E. coli* isolates from healthy humans, and to analyse the surrounding regions and their possible inclusion into integrons.

Between March and October 2007, 105 faecal samples were collected from healthy humans (age range 3–85 years) living in two regions in the Centre and in the North of Spain, Madrid (23 samples) and La Rioja (82 samples), respectively. None of the individuals included in the study had been exposed to antimicrobial agents or to a hospital environment in the 3 months prior to sample recovery. Samples were seeded onto Levine agar plates supplemented with cefotaxime (CTX, 2 μg/mL). After incubation at 37°C for 48 h, two colonies per sample showing *E. coli* morphology were recovered, identified by classical biochemical methods and by species-specific PCR (amplification of *uidA* gene), and screened for ESBL production according to CLSI criteria [14].

All ESBL-positive *E. coli* isolates obtained from this screen were included in this study for further characterization. Susceptibility testing to 17 antimicrobials [ampicillin, amoxicillin-clavulanic acid, cefoxitin, CTX, ceftazidime (CAZ), imipenem, aztreonam, gentamicin, streptomycin, kanamycin, nalidixic acid, ciprofloxacin, trimethoprim-sulfamethoxazole, sulphonamides, tetracycline, rifampicin and chloramphenicol] was performed using the disc-diffusion and agar dilution methods [14]. Genes encoding CTX-M, SHV, TEM, OXA and CMY type β-lactamases, and the genetic environment of $bla_{\text{CTX-M}}$ genes, were analysed by gene-specific PCR and DNA sequencing [15]. The presence of class 1, 2 and 3 integrons, as well as the characterization of the gene cassette arrangements were studied by PCR and DNA sequencing [16]. The presence of genes associated with tetracycline [*tet*(A)-*tet*(E), *tet*(M)], streptomycin [*aadA*], sulphonamides [*sul1*, *sul2*, *sul3*], kanamycin [*aph(3′)-Ia*, *aph(3′)-IIa*], and gentamicin resistance [*aac(3)-II*, *aac(3)-IV*] were also analysed [16]. In addition, amino acid changes in GyrA and ParC proteins were studied by PCR and sequencing of the corresponding genes in quinolone-resistant isolates [16]. The identification of the major phylogenetic groups of ESBL-positive isolates was determined by PCR [17].

**TABLE 1. Characteristics of the seven extended-spectrum β-lactamase (ESBL)-positive *Escherichia coli* isolates recovered from faecal samples of healthy humans**

| *E. coli* isolate[a] | Type of ESBL | Phylogenetic group | MIC (μg/mL) | | Resistance to non-β-lactams | Other genes detected[b] | Class 1 integron | | Amino acid changes in[c] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | CTX | CAZ | | | *intI1* | Gene cassettes | GyrA | ParC |
| Pn244-L | CTX-M-1 | $B_1$ | >128 | 4 | STR[d] | *aadA* | – | – | | |
| Pn248-L | CTX-M-1 | D | >128 | >128 | STR[d] | *aadA* | – | – | | |
| Pn215-L | CTX-M-8 | A | >128 | 1 | TET–NAL | *tet* (B) | – | – | S83L | Wild-type |
| Pn219-L | CTX-M-14a | $B_2$ | >128 | 2 | TET–STR–SUL | *tet* (B), *sul2* | – | – | | |
| Pn138-M | CTX-M-14b | D | >128 | >128 | STR[d]–SXT–SUL | *sul1* | –[e] | *dfrA16, aadA2* | | |
| Pn137-M | CTX-M-32 | $B_2$ | >128 | >128 | STR–KAN–GEN–SUL–NAL–CIP | *aph(3′)-Ia, aac(3)-II, sul3* | + | *estX, psp,aadA2* | S83L + A84P | S80I |
| Pn357-L | TEM-52 | A | >128 | 8 | STR[d] | – | – | – | | |

CTX, cefotaxime; CAZ, ceftazidime; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; SUL, sulphonamides; SXT, trimethoprim-sulfamethoxazole, TET, tetracycline.
[a]The last letter of the isolate names shows the geographical origin of the samples: L, La Rioja and M, Madrid.
[b]Detected outside integron structure.
[c]Amino acid changes in GyrA and ParC have been studied only in quinolone-resistant isolates.
[d]Intermediate category according to the CLSI standards.
[e]The *intI1* gene is interrupted at the 3′ end by the insertion of the IS*26* element.

ESBL-producing *E. coli* isolates were detected in seven of the 105 analysed faecal samples (6.6%). Two *E. coli* isolates were recovered per positive sample, but only one of each pair was kept for further studies due to the identical antimicrobial resistance phenotypes and ESBL genes exhibited by both isolates. Table 1 shows the characteristics of the isolates recovered from these positive samples. All of them were found to be unrelated clonally when studied by repetitive sequence-based PCR (data not shown). They presented MIC values for CTX of >128 μg/mL and for CAZ from 1 to >128 μg/mL. The ESBL genes found were $bla_{\text{CTX-M-14}}$ (in two isolates), $bla_{\text{CTX-M-1}}$ (in two), $bla_{\text{CTX-M-32}}$ (in one), $bla_{\text{CTX-M-8}}$ (in one), and $bla_{\text{TEM-52}}$ (in one). The $bla_{\text{CTX-M}}$ genes detected in these isolates are included in three CTX-M groups: $bla_{\text{CTX-M-14}}$ in the CTX-M-9-group, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-32}}$ in the CTX-M-1-group, and $bla_{\text{CTX-M-8}}$ in the CTX-M-8-group. All PCR tests performed to detect the presence of other *bla* genes were negative.

According to previous reports, ESBLs of the CTX-M-8 group are very unusual in Europe and have been previously detected in *E. coli* only in the UK, as the CTX-M-40 variant [18], and never before in Spain.

An increase in the rate of ESBL-positive *E. coli* in commensal microbiota of healthy children has been previously observed in Latin America (0.1% in 2002 vs. 1.7% in 2005), and diversification of the type of CTX-M β-lactamases has also been reported [13]. In 2003, levels of faecal colonization by ESBL-positive *E. coli* isolates of 2.4% and 3.7% were found in Lebanon [12] and Spain [4], respectively. The higher rate found in our study (6.6% in 2007) is, however, less than that recently detected in non-related healthy individuals in the South of Spain (7.4%) [6] or in Saudi Arabia (13.1%) [5], and less than the 16.7% and 27.4% detected in household contacts of community patients in two further studies in Spain [6,11]. The prevalence of faecal carriage of ESBL-positive *E. coli* strains has significantly increased in recent years, suggesting that this is an emerging problem that is worsening with time.

Different resistance phenotypes and genotypes were identified among the *E. coli* isolates studied. The *tet*(B), *aadA, sul1*/*sul2*/*sul3*, *aph(3′)-Ia* and *aac(3)-II* genes were found in most of the tetracycline-, streptomycin-, sulfamethoxazole-, kanamycin- and gentamicin-resistant ESBL-producing isolates. Only the CTX-M-32-producing isolate showed ciprofloxacin resistance and presented an unusual amino acid change in GyrA (Ser83Leu and Ala84Pro), in addition to a change in ParC (Ser80Ile). The $B_2$ or D phylogenetic groups comprised four of the isolates, and the remaining three isolates were assigned to the A or $B_1$ phylogenetic groups (Table 1).

The region surrounding the $bla_{\text{CTX-M}}$ genes detected in our isolates is shown in Fig. 1. The $bla_{\text{CTX-M-14a}}$ genetic variant, flanked by IS*Ecp1* and IS*903* sequences, was found in one of the isolates (Pn219), similar to isolates described in previous reports [9,15,21]. Moreover, another isolate (Pn138) possessed the unusual $bla_{\text{CTX-M-14b}}$ variant, included into an In60 integron with a *dfrA16* and *aadA2* gene cassette combination in its variable region and with the *intI1* gene truncated at the 3′ end by the insertion of the IS*26* element. The integron-associated $bla_{\text{CTX-M-14b}}$ variant has been described very recently in four human clinical *E. coli* isolates in Spain and in Korea [19,20], but has not previously been identified from faecal *E. coli* isolates from healthy humans.

The IS*26*/IS*Ecp1* and *orf477* sequences were detected on either side of the $bla_{\text{CTX-M-1}}$ gene in two of our *E. coli*

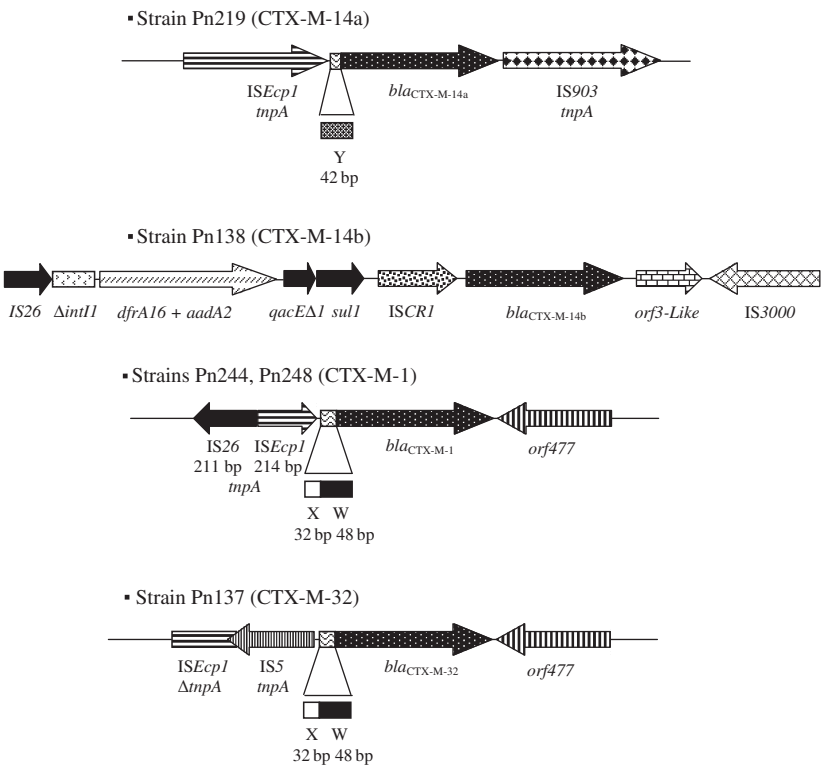


**FIG. 1.** Genetic environments of the $bla_{CTX\text{-}M}$ genes detected among the extended-spectrum $\beta$-lactamase-positive *Escherichia coli* strains studied (the intergenic X, Y and W regions have been previously reported [9,21]).

isolates (Pn244 and Pn248); a similar arrangement has been described by others [9,21]. In another isolate (Pn137), the IS*Ecp1* truncated transposase and the IS*5* sequence were detected upstream of the $bla_{CTX\text{-}M\text{-}32}$, and the *orf477* downstream of this *bla* gene, as previously reported [22]. The surrounding region of the $bla_{CTX\text{-}M\text{-}8}$ gene could not be identified. The $bla_{CTX\text{-}M\text{-}32}$-positive isolate (Pn137) harboured an unusual class 1 integron with the *estX*, *psp*, *aadA2* arrangement in its variable region (Table 1), although we could not demonstrate the inclusion of the ESBL gene inside the integron structure.

In conclusion, a moderate prevalence and high diversity of ESBLs were detected in faecal *E. coli* isolates of healthy humans, mainly of the CTX-M type. This is remarkable as the first detection of CTX-M-8 group determinants in Spain, and of the $bla_{CTX\text{-}M\text{-}8}$ gene in Europe. The community could be a reservoir of ESBL-producing *E. coli* isolates. This is the first time that the $bla_{CTX\text{-}M\text{-}14}$ gene has been found included into the In60 integron structure in commensal *E. coli* from healthy humans.

## Acknowledgements

This work has been presented in part in the 18th ECCMID Congress (Barcelona, Spain, 2008).

## Transparency Declaration

This work was partially supported by the Project SAF2006-14207-C02 from the Ministry of Education and Science of Spain. L.V. was supported by a fellowship from the Spanish Ministry of Education and Science (SAF2006-14207-C02-01) and S.S. by a fellowship from the Gobierno de La Rioja, Spain (Colabora 2007/15). No conflicting or dual interests exist in this study.

## References

1. Cantón R, Novais A, Valverde A *et al.* Prevalence and spread of extended-spectrum $\beta$-lactamase-producing *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* 2008; 14: 144–153.
2. Livermore DM, Cantón R, Gniadkowski M *et al.* CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* 2007; 59: 165–174.
3. Castillo García FJ, Seral García C, Pardos De la Gándara M, Millán Lou MI, Pitart Ferré C. Prevalence of faecal carriage of ESBL-producing *Enterobacteriae* in hospitalized and ambulatory patients during two non-outbreak periods. *Eur J Clin Microbiol Infect Dis* 2007; 26: 77–78.
4. Valverde A, Coque TM, Sánchez-Moreno MP, Rollán A, Baquero F, Cantón R. Dramatic increase in prevalence of faecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* during nonoutbreak situations in Spain. *J Clin Microbiol* 2004; 42: 4769–4775.
5. Kader AA, Kumar A, Kamath KA. Fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella*

*pneumoniae* in patients and asymptomatic healthy individuals. *Infect Control Hosp Epidemiol* 2007; 28: 1114–1116.
6. Rodríguez-Baño J, López-Cerero L, Navarro MD, de Alba PD, Pascual A. Faecal carriage of extended-spectrum β-lactamase-producing *Escherichia coli*: prevalence, risk factors and molecular epidemiology. *J Antimicrob Chemother* 2008; 62: 1142–1149.
7. Briñas L, Moreno MA, Teshager T *et al.* Monitoring and characterization of extended-spectrum β-lactamases in *Escherichia coli* strains from healthy and sick animals in Spain in 2003. *Antimicrob Agents Chemother* 2005; 49: 1262–1264.
8. Carattoli A. Animal reservoirs for extended-spectrum β-lactamase producers. *Clin Microbiol Infect* 2008; 14 (suppl): 117–123.
9. Jouini A, Vinué L, Slama KB *et al.* Characterization of CTX-M and SHV extended-spectrum beta-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. *J Antimicrob Chemother* 2007; 60: 1137–1141.
10. Mesa RJ, Blanc V, Blanch AR *et al.* Extended-spectrum beta-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage). *J Antimicrob Chemother* 2006; 58: 211–215.
11. Valverde A, Grill F, Coque TM *et al.* High rate of intestinal colonization with extended spectrum β-lactamases producing organisms in household contacts of infected community patients. *J Clin Microbiol* 2008; 46: 2796–2799.
12. Moubareck C, Daoud Z, Hakime NI *et al.* Countrywide spread of community- and hospital-acquired extended-spectrum β-Lactamase (CTX-M-15)-producing *Enterobacteriaceae* in Lebanon. *J Clin Microbiol* 2005; 43: 3309–3313.
13. Pallecchi L, Bartoloni A, Fiorelli C *et al.* Rapid dissemination and diversity of CTX-M extended-spectrum beta-lactamase genes in commensal *Escherichia coli* isolates from healthy children from low-resource settings in Latin America. *Antimicrob Agents Chemother* 2007; 51: 2720–2725.
14. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing, 17th informational supplement Approved standard M100-S17*. Wayne, PA: Clinical and Laboratory Standards Institute, 2007.
15. Vinué L, Lantero M, Sáenz Y *et al.* Characterization of extended-spectrum β-lactamases and integrons in *Escherichia coli* isolates in a Spanish hospital. *J Med Microbiol* 2008; 57: 916–920.
16. Sáenz Y, Briñas L, Domínguez E *et al.* Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. *Antimicrob Agents Chemother* 2004; 48: 3996–4001.
17. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555–4558.
18. Hopkins KL, Deheer-Graham A, Threlfall EJ, Batchelor MJ, Liebana E. Novel plasmid-mediated CTX-M-8 subgroup extended-spectrum β-lactamase (CTX-M-40) isolated in the UK. *Int J Antimicrob Agents* 2006; 27: 572–575.
19. Bae IK, Lee YN, Lee WG, Lee SH, Jeong SH. Novel complex class 1 integron bearing an ISCR1 element in a *Escherichia coli* isolate carrying the $bla_{CTX-M-14}$ gene. *Antimicrob Agents Chemother* 2007; 51: 3017–3019.
20. Navarro F, Mesa RJ, Miró E, Gómez L, Mirelis B, Coll P. Evidence for convergent evolution of CTX-M-14 ESBL in *Escherichia coli* and its prevalence. *FEMS Microbiol Lett* 2007; 273: 120–123.
21. Eckert C, Gautier V, Arlet G. DNA sequence analysis of the genetic environment of various $bla_{CTX-M}$ genes. *J Antimicrob Chemother* 2006; 57: 14–23.
22. Cartelle M, Tomas MM, Molina F, Moure R, Villanueva R, Bou G. High-level resistance to ceftazidime conferred by a novel enzyme, CTX-M-32, derived from CTX-M-1 through a single Asp240-Gly substitution. *Antimicrob Agents Chemother* 2004; 48: 2308–2313.

# Seasonal variation of *Pneumocystis jirovecii* infection: analysis of underlying climatic factors

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## Abstract

*Pneumocystis jirovecii* causes severe pneumonia (PCP) in immunocompromised patients. Seasonal changes of PCP incidence may be associated with climate changes. In this first study using multiple linear regression statistics to assess monthly climatic data and *Pneumocystis*, PCP incidence was positively correlated with mean temperature, but not with rainfall or wind strength.

**Keywords:** Climate, environment and public health, epidemiology, incidence, infection, meteorological factors, *Pneumocystis jirovecii*, *Pneumocystis* pneumonia

**Original Submission:** 7 August 2008; **Revised Submission:** 13 October 2008; **Accepted:** 24 October 2008
Editor: S. Cutler
**Article published online:** 9 June 2009

*Clin Microbiol Infect* 2009; **15:** 957–960
10.1111/j.1469-0691.2009.02804.x

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*Pneumocystis jirovecii* pneumonia (PCP) is one of the most frequent and serious opportunistic infections. However, the modes of transmission of PCP remain unknown. Air-borne